METHODS OF DETERMINING GLUCOSE AND FRUCTOSE IN CORN TISSUES[1]

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(WITH TWO FIGURES)

Introduction

In a series of studies on the physiology of corn, considerable attention has been given to the concentration of sugars in the different tissues of the plant, especially in relation to seasonal development. In these studies the total sugar content was differentiated into free reducing sugars and sucrose. It seemed desirable to carry the investigations a step further and determine the proportions of glucose and fructose which make up the free reducing sugars. Due to the similarity in chemical properties of these two sugars, however, procedures have not been standardized for the determination of either one of them in the presence of the other and sucrose in plant tissues. It consequently was necessary to make a preliminary survey of the available methods and to work out procedures which might be satisfactory for use with samples of corn tissues.

Three of the general methods which have been used for determining glucose and fructose in mixtures of glucose, fructose, and sucrose are: (1) the oxidation of glucose in alkaline solution with iodine, (2) the oxidation of fructose with Nyns' solution, a cupro-potassium carbonate solution, and (3) the method of analyzing a mixture of three sugars by determining the combined reducing power, polarization, and the direct determination of sucrose.

Materials and methods

In our studies of corn the tissue to be sampled was prepared by passing it through a food grinder, and it was desired to develop methods which might be applicable to two kinds of sampling. In one, the "Expressed sap method," the sap was expressed from 100 gm. of the ground tissue in a laboratory hydraulic press at a pressure of 5000 lb. per sq. in. Fifty-ml. aliquots of the sap were preserved by boiling with about 150 ml. of 95 per cent. alcohol and then making up to 200 ml. when cooled to room temperature. From determinations of the weight, total solids content, and specific gravity of the sap and the total moisture content of the tissue, the quantities of sugars found in the sap were calculated to percentages of fresh tissue.

[1] Based on investigations co-operative between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

The second kind of sample was obtained by the standard method of extracting 50 gm. of the ground tissue with 80 per cent. alcohol in a Soxhlet extractor. As the first method is quicker and simpler, the primary purpose of the extraction method was to check the accuracy of the expressed sap method.

Iodometric determination of glucose

An excellent review of the literature and a critical study of the factors involved in the oxidation of aldose sugars, such as glucose, by iodine in alkaline solution has been reported recently by Kline and Acree (**4**). The reaction is usually conceived of as occurring as follows:

$$RCHO + 3NaOH + I_2 \rightarrow RCOONa + 2NaI + 2H_2O$$

Determinations of glucose were made on portions of the same cleared aliquots of the alcoholic solutions as were used for determining the reducing power by means of Fehling's solution. In the clearing, 25 ml. of the alcohol-preserved sap sample, or 50 ml. of the alcoholic extract, were pipetted into a 100-ml. volumetric flask and the alcohol driven off on a steam plate. The syrupy residue was taken up in about 50 ml. of distilled water. To this, 0.5 ml. of a saturated solution of neutral lead acetate was added. The contents of the flask were made up to volume, shaken, and filtered through a folded filter. Sufficient potassium oxalate was added to precipitate the excess lead and the solution again filtered. It was found that any of the solutions which retained some color after undergoing this clearing process contained material which was oxidized by iodine, thus introducing considerable error. Such material, however, was found to be removed by treating the solution with about 1 gm. of decolorizing carbon (Baker and Adamson, Code BA 1551 bought from General Chemical Company) and filtering again. The effect of decolorizing with carbon on the indicated glucose contents of two solutions representing leaf tissues is shown in table I. That this effect of the carbon is not due to absorption of glucose is shown by the results with a pure glucose solution given in the same table.

The oxidation of the glucose was carried out as follows: a 25-ml. aliquot of the cleared and decolorized sugar solution was pipetted into a 250-ml. iodine flask and from 5 to 10 ml. of an approximately N/10 iodine solution run in from a burette. The quantity of iodine used depended upon the quantity of glucose present, since too great an excess causes over-oxidation. Five ml. were used for samples containing up to about 40 mg. of glucose (all samples of leaf tissue), and 10 ml. for those containing more (stem, tassel, and ear samples). A 10 per cent. sodium carbonate solution was added at the rate of 1.5 ml. for each 5 ml. of iodine and the flask placed in the dark for 15 minutes. Observations indicated that the oxidation was

TABLE I

EFFECT OF DECOLORIZING CLEARED SOLUTIONS ON THE QUANTITY OF IODINE USED

SAMPLE	TREATMENT	N/10 I USED	GLUCOSE
		ml.	*per cent.*
1. Leaf tissue	Without carbon	2.90	1.97
	Carbon added	2.10	1.43
2. Leaf tissue	Without carbon	3.00	2.04
	Carbon added	2.10	1.43
3. Glucose solution	Without carbon	2.55	1.73
	Carbon added	2.55	1.73

probably complete in 5 minutes, but, since there was no evidence of increased oxidation at the end of 15 minutes, that length of time was used as being more convenient. About 2 ml. of a freshly prepared starch solution was added and the excess iodine titrated with N/20 sodium thiosulphate. About 0.5 ml. of dilute sulphuric acid was added and the iodine thus released from combination as hypoiodite or iodate was titrated with the thiosulphate. Alternate additions of the dilute sulphuric acid and the N/20 sodium thiosulphate were made until no more iodine appeared on the addition of acid. This alternate addition of small quantities of acid and thiosulphate is probably much to be preferred to the addition of a rather large excess of acid at the start of the titration in working with solutions which contain sucrose. Otherwise, hydrolysis of the sucrose by the acid might provide glucose which would be immediately oxidized by the iodine present.

The weight of glucose oxidized by the iodine is calculated on the basis that 1 ml. N/20 iodine solution = 0.0045 gm. glucose.

DETERMINATION OF FRUCTOSE BY NYNS' METHOD

The determination of fructose by the use of Nyns' solution was carried out according to the procedure described by ZERBAN and SATTLER (**8**).

Determinations were made on aliquots of the same cleared and decolorized solutions used for the determination of glucose. It was found that the decolorizing step, however, was not necessary since the same results were obtained with or without the use of carbon.

To 10 ml. of the cleared solution in a 100-ml. centrifuge tube was added 10 ml. of distilled water and 50 ml. of Nyns' solution. The mixture was kept in a constant temperature water bath at 48.5–49.0° C. for 2.5 hours. The tube was then removed and centrifuged for 5 minutes. The supernatant liquid was carefully poured off, about 15 ml. of distilled water added from a wash bottle, and the tube again centrifuged. The supernatant

liquid was poured off and the copper oxide determined by dissolving in Bertrand's solution (20 per cent. sulphuric solution saturated with ferric sulphate) and titrating with N/20 potassium permanganate.

Although NYNS (5) claimed that when the factors of time, temperature, and concentration were closely guarded, the reagent showed a selective reactivity towards fructose in the presence of glucose and sucrose, this has not been substantiated by later workers, JACKSON (3), SCHUETTE and TERRILL (6), and ZERBAN and SATTLER (8). The last workers found, however, that with quantities of fructose ranging from 12 to 30 mg., and with about an equal quantity of glucose, approximately 13 mg. of glucose were required to reduce a quantity of copper equal to that reduced by 1 mg. of fructose. Preliminary determinations showed that the percentage of glucose in the free reducing sugars of corn tissues ranged from 50 to 70 per cent. The fructose-copper equivalents used in this study accordingly were determined on solutions containing 60 per cent. glucose and 40 per cent. fructose. The fructose-copper values are given in table II.

TABLE II

FRUCTOSE-COPPER EQUIVALENTS OF SOLUTIONS CONTAINING, IN ADDITION TO THE QUANTITIES OF FRUCTOSE STATED, 1.5 TIMES AS MUCH GLUCOSE

FRUCTOSE	CU
mg.	*mg.*
5	13.6
10	32.5
15	51.1
20	70.5
25	88.0
30	107.5
40	143.5

It was recognized that for proportions other than those mentioned above the quantity of copper reduced by 13 mg. of glucose might vary slightly from that reduced by 1 mg. of fructose. The sugar solutions from the samples of corn tissues, however, contained only from 2 to 18 mg. of fructose in the 10-ml. aliquots used for the fructose determination; hence, it was assumed that in solutions with such a limited range in concentration the error introduced by variations in this factor would be overshadowed by other errors.

For convenience in calculating results the chart shown as figure 1 was prepared. In this chart the abscissae represent the percentages of free reducing sugars, and the ordinates represent the milligrams of copper

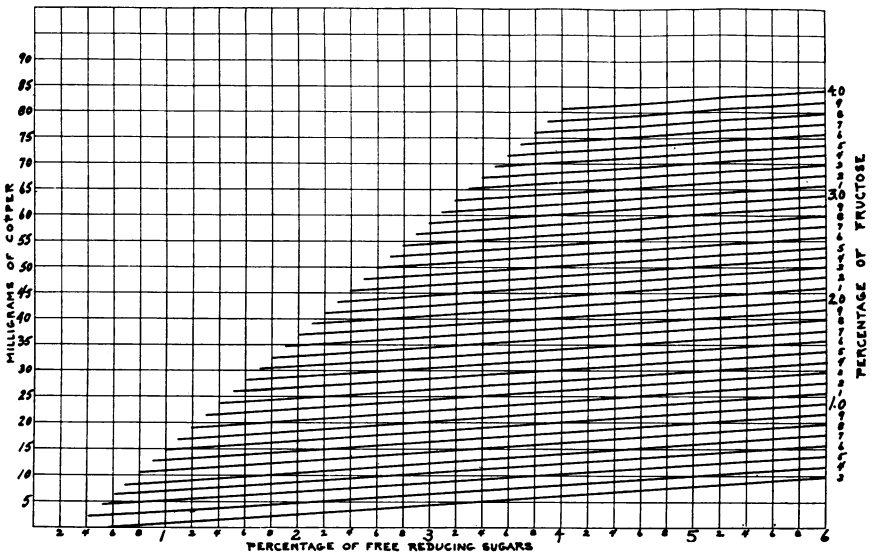

FIG. 1. Chart for calculation of percentage of fructose from determinations of percentage of free reducing sugars and milligrams of copper reduced from Nyns' solution.

reduced from Nyns' solution. By projecting the point of intersection of ordinate and abscissa for a given sample to the right hand margin, in a direction parallel to the sloping lines, the percentage of fructose may be read directly. This particular chart, of course, holds good only within the limits of the exact conditions used in this study with respect to dilution factors, etc.

POLARIZATION METHOD

A method of calculating the percentages of glucose, fructose, and sucrose in a mixture of the three sugars from determinations of reducing power, polarization, and the direct determination of sucrose has been described by BROWNE (**1**).

As modified for use with samples of corn tissues the calculation was based upon the following determinations: (1) the quantity of free reducing sugars by reduction of Fehling's solution; (2) the quantity of sucrose as determined by the additional reducing power after the inversion of sucrose with invertase; and (3) the polarization of the sugar solution.

The free reducing power was calculated as invert sugar and no further correction was made for the difference between the reducing ratios of glucose and fructose, since the concentrations of reducing sugars were relatively low and the range in proportions of glucose and fructose rather narrow (50 to 70 per cent. glucose).

The samples were prepared for polarization in the following manner: 100 ml. of the alcoholic expressed sap sample or 250 ml. of the alcoholic extract were transferred to a beaker and the alcohol driven off on a steam plate. The syrupy residue was taken into solution and washed into a 25-ml. volumetric flask with about 20 ml. of distilled water; 0.5 ml. of a saturated solution of neutral lead acetate then was added and the contents made up to volume and shaken. The solution was filtered through a good grade of filter-paper and the clear filtrate polarized in a 200-mm. tube, using a Schmidt-Haensch saccharimeter. The source of light was a microscope lamp containing a 400-watt stereopticon bulb.

The calculated polarization due to the sucrose present was subtracted from the observed polarization, thus giving the polarization due to the mixture of glucose and fructose. The proportions of glucose and fructose, expressed as percentage of glucose, were then obtained by use of a chart such as is illustrated in figure 2. On this chart is represented the rotation

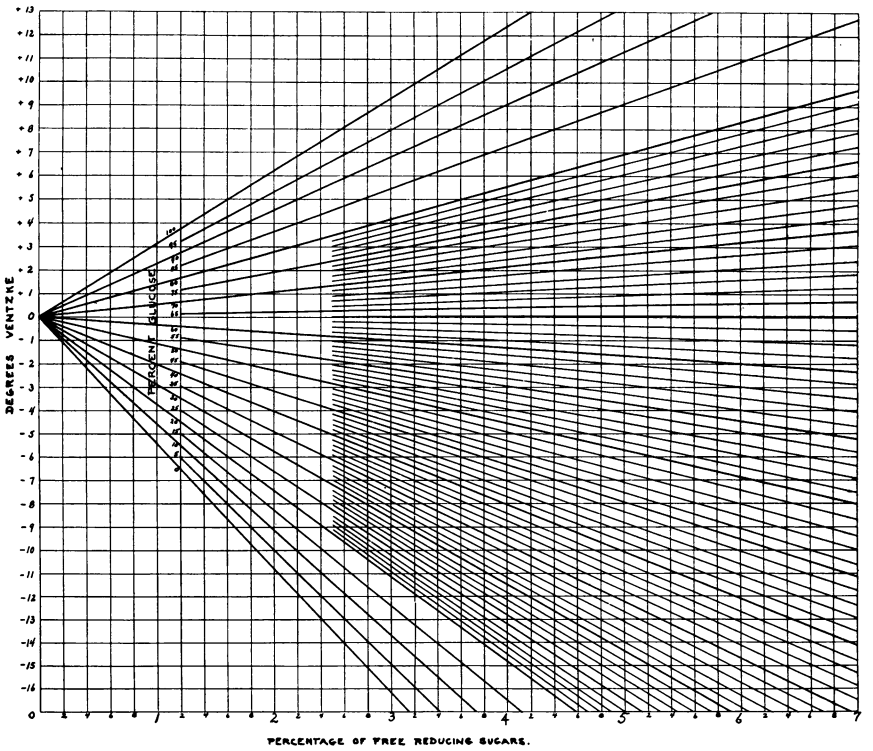

FIG. 2. Chart for calculation of percentage of glucose in free reducing sugars from determinations of free reducing sugars and their rotation.

in degrees Ventzke of all possible proportions of glucose and fructose in sugar solutions containing up to 7 per cent. free reducing sugars, calculated from their specific polarizations. On this chart the ordinates represent the rotation in degrees Ventzke due to the free reducing sugars and the abscissae represent the percentages of free reducing sugars. At the point of intersection of ordinate and abscissa for a given sample the proportion of glucose and fructose was read, expressed as percentage of glucose in the free reducing sugars.

EXAMPLE.—A sample containing 2.38 per cent. free reducing sugars and 6.02 per cent. sucrose was found to have a rotation of + 22.8° V. Since the rotation of a 6.02 per cent. sucrose solution is + 23.1° V., the 2.38 per cent. of free reducing sugars has a rotation of − 0.3° V. As shown by the chart, figure 2, 62.2 per cent. of the free reducing sugars was glucose, and therefore the sample contained 1.48 per cent. glucose and 0.90 per cent. fructose.

ANALYSIS OF PURE SUGAR SOLUTIONS

As a check on the accuracy of the three methods of determining glucose and fructose a series of 10 samples containing known quantities of glucose, fructose, and sucrose was analyzed by each of the methods. The concentrations of sugars in these samples were representative of those actually found in samples of corn tissues. The sugars were Pfanstiehl brand prepared by the Special Chemicals Co.

The samples were prepared in such a way as to simulate the preparation of the expressed sap samples previously described. The quantities of sugars for each sample were weighed out, placed in 200-ml. graduated prescription bottles, and 50 ml. of distilled water added to dissolve the sugars. The bottles were then filled nearly to the 200-ml. mark with 95 per cent. alcohol and heated in a water-bath for 30 minutes. After cooling to room temperature they were filled to the 200-ml. mark, thoroughly shaken, and set aside for analysis. In analyzing these solutions exactly the same procedures were followed as with the expressed sap samples, including the addition of neutral lead acetate and decolorizing carbon.

The composition of the samples as prepared and found by analysis is reported in table III. The determinations made were: reducing sugars before and after inversion of sucrose with invertase as measured by reduction of Fehling's solution; glucose by the iodine method; fructose by Nyns' solution; and the proportion of glucose and fructose by the polarization method, the results of the last determination being reported as glucose.

The mean quantity of glucose present in the samples was 1.71 per cent. while that indicated by the iodine method was 1.735 and by the polarimeter method was 1.728. The average quantity of fructose indicated was 1.15

TABLE III

COMPOSITION (GRAMS PER 100 ml.) OF PREPARED SOLUTIONS OF PURE SUGARS

SAMPLE	AS PREPARED	COMPOSITION OF SAMPLES AS FOUND BY THE METHOD OF ANALYSIS INDICATED				
		GLUCOSE		FRUCTOSE	FEHLING'S SOLUTION	
		IODINE	POLARIZATION	NYNS'	FREE REDUCING SUGARS	SUCROSE
no.		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	Glucose 0.60 Fructose 0.40 Sucrose 0.50	0.57	0.60	0.42	1.01	0.54
2	Glucose 1.20 Fructose 0.80 Sucrose 0.50	1.20	1.20	0.78	1.97	0.51
3	Glucose 1.20 Fructose 0.80 Sucrose 1.50	1.19	1.17	0.80	1.97	1.58
4	Glucose 1.50 Fructose 1.00 Sucrose 0.50	1.58	1.52	0.96	2.45	0.54
5	Glucose 1.50 Fructose 1.00 Sucrose 1.00	1.55	1.49	0.98	2.47	1.05
6	Glucose 1.50 Fructose 1.00 Sucrose 2.00	1.54	1.49	1.01	2.47	2.03
7	Glucose 1.80 Fructose 1.20 Sucrose 1.00	1.88	1.80	1.23	2.95	1.08
8	Glucose 2.40 Fructose 1.60 Sucrose 6.50	2.38	2.55	1.66	4.06	6.34
9	Glucose 2.70 Fructose 1.80 Sucrose 2.50	2.70	2.70	1.78	4.39	2.49
10	Glucose 2.70 Fructose 1.80 Sucrose 5.00	2.76	2.76	1.84	4.47	4.97
	Mean Glucose 1.71 Fructose 1.14	1.735	1.728	1.15		

per cent. whereas the samples actually contained 1.14 per cent. This close agreement, both as to means and individual samples obtained under the conditions of the experiment, indicated that all three methods might be expected to give results well within the limits of accuracy desirable with samples of corn tissues.

Comparison of methods with corn tissues

The three methods were compared on corn tissues during the season of 1930. The variety of corn used was Burr-Leaming, a double cross. For convenience in sampling, the plant was divided into leaves and stem, and when the plants attained a height of approximately 120 cm. the stem was divided into upper, middle, and lower parts.

The sugar content of the samples ranged from leaf tissues containing 0.9 per cent. to stem tissues containing 5.5 per cent.

Expressed sap and alcoholic extraction samples as previously described were used. The results obtained by each of the methods were expressed as percentages of glucose on a fresh weight basis. In the case of Nyns' method the data were calculated to this basis by subtracting the quantity of fructose found from the free reducing sugars, the difference being regarded as glucose.

Single determinations were made on each of the duplicate samples taken from each lot of corn tissue. This naturally tended to make the differences between the comparable values relatively large since they reflect not only the variation due to analytic technic but also that due to sampling. The dependability of the averages representing the corn was increased by this procedure.

Results

The three methods were compared with regard to: (1) agreement in determining the mean glucose content of all samples; (2) agreement in determining the glucose content of different kinds of tissue in varying stages of development; and (3) the relative precision of the different methods in determining the glucose in duplicate samples.

Expressed sap samples

The percentages of glucose in samples of expressed sap as determined by the different methods are shown in table IV. The mean of the 30 samples was 1.767 per cent. for the iodine method, 1.780 per cent. for Nyns' method, and 1.919 per cent. for the polarization method. The significance of the differences among these means was tested, using Fisher's (**2**) formula, $t = \frac{\overline{x} - \overline{x}_1}{s}\sqrt{n}$, and the probability tables given by Student (**7**). Although the difference between means based on the iodine and Nyns' methods was only 0.013 per cent. and well might be due to chance, the odds

TABLE IV

PERCENTAGES OF GLUCOSE IN TWO KINDS OF SAMPLES OF CORN TISSUES AS DETERMINED BY THREE METHODS

Date of sampling	Tissue	Expressed sap samples Method						Alcoholic extraction samples Method					
		Iodine		Nyns'		Polarization		Iodine		Nyns'		Polarization	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
July 7	Leaves	0.69	0.62	0.54	0.52	0.61	0.59	0.73	0.73	0.49	0.48	0.64	0.59
	Stems	1.26	1.23	1.24	1.17	1.21	1.19	.....	.....	.....	.....	.....	.....
14	Leaves	0.91	0.86	0.69	0.68	0.71	0.71	.....	.....	.....	.....	.....	.....
	Stems	1.71	1.62	1.71	1.62	1.81	1.77	.....	.....	.....	.....	.....	.....
22	Leaves	0.98	0.89	0.93	0.86	1.24	1.13	0.76	0.79	0.87	0.85	1.10	1.14
	Stems	2.01	1.94	2.16	1.87	2.43	2.32	1.90	2.10	2.03	1.99	2.30	2.32
29	Leaves	0.91	0.86	1.01	0.98	0.99	0.98	.....	.....	.....	.....	.....	.....
	Upper stems	2.79	2.63	3.01	2.78	2.86	2.81	.....	.....	.....	.....	.....	.....
	Lower stems	2.40	2.36	2.65	2.62	2.69	2.58	.....	.....	.....	.....	.....	.....
Aug. 5	Leaves	1.12	1.12	1.15	1.08	1.21	1.15	1.24	1.20	1.00	1.02	1.06	1.11
	Upper stems	2.70	2.67	2.82	2.72	3.00	2.89	.....	.....	.....	.....	.....	.....
	Middle stems	2.33	2.27	2.42	2.40	2.69	2.61	.....	.....	.....	.....	.....	.....
	Lower stems	2.38	2.35	2.45	2.33	2.63	2.53	2.19	2.19	2.46	2.48	2.35	2.40
11	Leaves	1.15	1.13	1.14	1.09	1.21	1.12	1.13	1.12	1.18	1.16	1.16	1.23
	Sheaths	2.28	2.28	2.30	2.18	2.41	2.39	.....	.....	.....	.....	.....	.....
	Upper stems	2.59	2.54	2.78	2.77	2.99	2.93	.....	.....	.....	.....	.....	.....
	Middle stems	2.54	2.49	2.67	2.65	2.82	2.81	.....	.....	.....	.....	.....	.....
	Lower stems	2.46	2.27	2.41	2.33	2.58	2.56	2.30	2.30	2.50	2.64	2.55	2.79
18	Leaves	1.64	1.38	1.38	1.36	1.73	1.58	1.53	1.63	1.30	1.46	1.29	1.40
	Upper stems	2.39	2.29	2.29	2.24	2.52	2.45	.....	.....	.....	.....	.....	.....
	Middle stems	2.22	2.17	2.13	2.07	2.33	2.28	.....	.....	.....	.....	.....	.....
	Lower stems	2.29	2.24	2.23	2.08	2.29	2.17	2.31	2.31	2.30	2.30	2.35	2.31
25	Leaves	1.01	0.97	0.94	0.87	0.91	0.87	0.89	0.88	0.90	0.86	0.85	0.83
	Upper stems	2.21	2.18	1.88	1.85	2.10	2.07	.....	.....	.....	.....	.....	.....
	Middle stems	1.85	1.82	2.06	2.03	2.28	2.28	.....	.....	.....	.....	.....	.....
	Lower stems	2.12	2.03	2.23	2.17	2.36	2.34	2.00	2.00	2.40	2.28	2.49	2.56
Sept. 8	Leaves	1.05	0.97	1.15	1.06	1.26	1.17	0.96	1.14	1.00	1.00	1.16	1.18
	Upper stems	1.27	1.26	1.43	1.41	1.64	1.60	.....	.....	.....	.....	.....	.....
	Middle stems	1.28	1.24	1.34	1.21	1.44	1.32	.....	.....	.....	.....	.....	.....
	Lower stems	1.39	1.38	1.35	1.30	1.48	1.48	1.45	1.38	1.48	1.46	1.46	1.48
	Mean	1.767		1.780		1.919		1.506		1.534		1.619	

being 3:1, the odds were infinite against random differences as large as those between the means determined by the polarization method on the one hand and those determined by the iodine method and Nyns' method on the other.

The product moment coefficients of correlation for glucose content in the different samples were: for the iodine and Nyns' methods, 0.984 ± 0.004; for the iodine and polarization methods, 0.980 ± 0.005; and for the Nyns' and polarization methods, 0.991 ± 0.002.

The standard error of a single result, $\sqrt{\frac{\Sigma d^2}{(m-1)(n-1)}}$ is used to measure the precision of the different methods. In this formula Σd^2 indicates the sum of the squared deviations of the duplicates from their mean for each of the n $(=30)$ samples and m is the number in the group, here the two of each pair. The standard errors were 0.060, 0.069, and 0.053 for the iodine, Nyns', and polarization methods, respectively.

It may be concluded from this comparison of the three methods as applied to expressed sap samples that the correlation is high enough to show conclusively that the methods could be used interchangeably for determining variations due to kind and stage of development of tissues; furthermore, the precision of each of the methods was satisfactory inasmuch as all the errors are less than four per cent. of the total glucose content. On the other hand, the results obtained by the polarization method were significantly higher than those by the iodine and Nyns' method, the latter two being in good agreement.

Alcoholic extraction samples

The applicability of the three methods to alcoholic extraction samples was determined on 13 samples of the series. The percentages of glucose by the different methods are included in table IV.

The means for the 13 samples were: 1.506 per cent. for the iodine method, 1.534 per cent. for Nyns' method, and 1.619 per cent. for the polarization method. The odds were 20:1 and 90:1 against random differences as large as those between the means for the polarization method on the one hand and the iodine method and Nyns' method on the other. The difference between the means for the iodine and Nyns' methods was 0.028 with odds of only 2:1 against random differences this large. In the alcoholic extraction samples the product moment coefficients of correlation for the Nyns' method with the iodine and the polarization methods were both 0.986 ± 0.005. That for determinations by the polarization and iodine methods was 0.959 ± 0.015. The standard errors were 0.061, 0.051, and 0.062 for the iodine, Nyns' and polarization methods, respectively.

The same general conclusions may be drawn from this comparison of the three methods applied to alcoholic extraction samples as were arrived

at with expressed sap samples; namely, that while the methods can be used interchangeably for determining variations due to kind and stage of development of corn tissues and also are satisfactory from the point of view of precision, the mean of the results obtained by the polarization method is significantly higher than those based on the iodine and Nyns' methods, the latter two being in good agreement.

Comparison of analyses of expressed sap and alcoholic extraction samples

With both kinds of samples the polarization method gave a mean glucose content which was significantly higher than those by the iodine and Nyns' methods; all three methods agreed reasonably with respect to the variation in samples due to differences in kind of tissue or seasonal development and also with respect to the precision in determining the glucose content in duplicate samples. Since the alcoholic extraction samples were primarily to check the accuracy of the results obtained by analyzing the expressed sap and then calculating the glucose content of the original tissue, it seemed desirable to make a direct comparison of the analyses of the 13 comparable lots of tissue for which data on both kinds of samples were available.

There were no important differences between the means for any method in the two kinds of samples. Such differences as there were well might have been due to chance, the odds not exceeding 4:1 in any comparison.

The product moment coefficients of correlation for glucose content in the two kinds of samples ranged from 0.984 to 0.996. These high correlations in connection with the close agreement of mean values are sufficient evidence that expressed sap samples may be used in place of alcoholic extraction samples without error.

Determination of free reducing sugars

The determinations of glucose by oxidation with iodine and of fructose with Nyns' solution appear to give reliable results. These methods then might well replace the usual determination of free reducing sugars with Fehling's solution in some investigations.

To test this possibility, the determinations of free reducing sugars with Fehling's solution in the series of 30 expressed sap samples were compared with the sum of glucose by the iodine method and fructose by Nyns' method. The data are given in table V.

The mean of the 30 samples was 3.140 per cent. as determined with Fehling's solution and 3.127 per cent. for the sum of glucose and fructose. The difference is only 0.013, and no larger than might be expected from random sampling in one out of every 4 trials.

TABLE V

FREE REDUCING SUGAR CONTENT OF EXPRESSED SAP FROM CORN TISSUE SAMPLES AS DETERMINED BY TWO METHODS

DATE OF SAMPLING	TISSUE	FEHLING'S SOLUTION	GLUCOSE, IODINE + FRUCTOSE, NYNS
		per cent.	*per cent.*
July 7	Leaves	0.920	1.045
	Stems	2.170	2.210
14	Leaves	1.135	1.335
	Stems	3.380	3.380
22	Leaves	1.525	1.565
	Stems	3.875	3.835
29	Leaves	1.560	1.450
	Upper stems	5.300	5.115
	Lower stems	4.735	4.480
Aug. 5	Leaves	1.955	1.960
	Upper stems	5.575	5.490
	Middle stems	4.590	4.480
	Lower stems	4.310	4.285
11	Leaves	1.865	1.890
	Sheaths	4.025	4.065
	Upper stems	5.220	5.010
	Middle stems	4.650	4.505
	Lower stems	3.880	3.875
18	Leaves	2.170	2.310
	Upper stems	4.125	4.200
	Middle stems	3.615	3.710
	Lower stems	3.500	3.610
25	Leaves	1.375	1.460
	Upper stems	3.255	3.585
	Middle stems	3.385	3.175
	Lower stems	3.610	3.485
Sept. 8	Leaves	1.880	1.785
	Upper stems	2.390	2.235
	Middle stems	2.030	2.015
	Lower stems	2.190	2.250
	Mean	3.140	3.127

There is a high correlation for the free reducing sugar contents as determined by the two methods ($r = 0.997$). These methods of determining total free reducing sugars accordingly are in excellent agreement in all ways.

Discussion

In the comparison of the three methods of determining glucose in the expressed sap and alcoholic extraction samples of corn tissues used in this

study it was shown that the polarization method gave results which were significantly higher than those obtained by either the iodine or Nyns' method. All attempts to discover the reason for this discrepancy have failed. The most likely source of error first appeared to be that occasioned by the defecation of materials with neutral lead acetate in the 25-ml. volumetric flask. The sugar concentration of the solution presumably is increased in direct proportion to the volume occupied by this precipitate. Moreover, as would be expected, the tendency of the polarization method to give higher results was not observed in measuring the recovery of pure sugars from solutions of known concentration. On the other hand, it would be expected that this tendency toward higher values would be most marked with those solutions which contained the largest quantities of precipitable material; namely, those representing leaf tissues. The data in table I, however, do not seem to substantiate this idea. Instead, those solutions having the higher percentages of sugars, the stem samples, are the ones which show the greatest discrepancies.

Polarization has the advantage of simplicity in technique and may well serve in preliminary studies. Moreover, the error obtained in the use of this method is not large, the difference between the means by polarization and the iodine methods being 0.142 per cent. and that between polarization and Nyns' 0.139 per cent. on a fresh weight basis, differences of less than 8 per cent. of the total glucose. The largest individual difference, that of 0.445 per cent., was found between the polarization and iodine methods on August 25 in middle stem tissue, containing about 2 per cent. of glucose.

The chief disadvantages of polarization are: (1) the large quantity of extract necessary to get a sufficient concentration of sugar for a saccharimeter reading; and (2) some extracts, particularly of leaf tissues, are so highly colored after clearing that it is extremely difficult to obtain readings with the saccharimeter even with a powerful source of light.

The oxidation of glucose by iodine is undoubtedly the most sensitive and rapid method of the three compared. Since iodine is readily absorbed or reduced by many materials contained in plant extracts, however, unusual care must be taken in the clearing and decolorizing processes. Also, the quantity of iodine solution used must be carefully regulated since too great an excess causes over-oxidation and with an insufficient excess there is under-oxidation.

The method of Nyns is much less sensitive than the iodine method and the conditions under which the oxidation is carried out fit the method well to routine analysis. Furthermore, the apparatus and most of the reagents required are those also used in the determination of reducing power by Fehling's solution. The most serious objection to the method is that the reagent is not selective for fructose, but also oxidizes glucose, making the

calculation quite complicated. It would seem, however, that this method will be most valuable when used with samples, such as apple tissue in which fructose is the predominating free reducing sugar. Moreover, the results obtained in this study indicate that with the relatively small quantities of sugar found in most plant tissues, and with a rather constant glucose-fructose ratio, the calculation need not be so complicated in arriving at analyses which are within the range of experimental error of such materials.

Summary

1. With pure sugar samples all three methods gave results which were well within the limit of accuracy desired for use with samples of corn tissues.

2. The iodine and Nyns' methods gave values for glucose which were in excellent agreement in every way when used with expressed sap and alcoholic extraction samples.

3. The results obtained by the polarization method with the corn samples were consistently higher, the mean values being about 8 per cent. higher. The precision of the method, however, was equal to the other two and probably has some value in preliminary studies.

4. Either expressed sap or alcoholic extraction samples could be used equally well with all three methods.

5. Free reducing sugars may be determined from the sum of glucose by iodine and fructose by Nyns' methods with the same degree of accuracy as directly by the reducing power with Fehling's solution.

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